SUPPLEMENTAL MATERIAL

Data S1.

Supplemental Methods

Detailed description of the used CMR technique (native T1 mapping) and histopathological analysis of endomyocardial biopsies for quantification of myocardial fibrosis.

Cardiovascular magnetic resonance (CMR) and image analysis

All CMR studies were performed on a 3-Tesla system (Magnetom® Verio, Siemens Healthineers, Erlangen, Germany) and included a stack of short-axis slices from the base of the heart to the apex using a cine steady-state free precession (SSFP) sequence (TR 2.9-3.0 ms, TE 1.5 ms, flip angle 60°, in plane resolution 1.3 x 1.3 x 6.0 mm, temporal resolution 25 phases, breath-hold). Native T1 mapping data were collected in the mid-ventricular short axis plane with a breath-hold modified Look-Locker inversion recovery (MOLLI) sequence (parameters: FOV 306 × 360 mm, TR/TE 4.9/1.2 ms, flip angle 35°, slice thickness 8 mm, voxel size 1.4 × 1.4 × 8 mm).

All analyses were performed with commercially available software (cmr42, Circle Cardiovascular Imaging, Calgary, Canada). LV endo- and epicardial contours were manually drawn in the short axis stack to measure end- diastolic and end-systolic volumes (LVEDV, LVESV) and LV mass was calculated by subtracting endocardial from epicardial volume at end-diastole and multiplying by 1.05 g/cm^3. All parameters were adjusted to body surface area (BSA). LV ejection fraction and stroke volume was calculated automatically from the volumes. T1 times were measured in the interventricular septum and within the entire LV myocardium on the midventricular short axis slice (**Figure 1C**).

Routine cardiac catheterization

All studies were carried out under sedation using a combination of midazolam, ketamine and propofol with the patients breathing spontaneously. The protocol included hemodynamic assessment with quantification of left and right end-diastolic pressures as well as pulmonary arterial pressures. Coronary angiograms were analyzed to determine the grade of coronary allograft vasculopathy (CAV) in each patient applying angiographic criteria according to the International Society for Heart and Lung Transplantation (ISHLT) guidelines.(10)

Histopathological analysis

At least three endomyocardial biopsies were taken from the right interventricular septum that were immediately fixed in formaldehyde for routine light microscopy examination. The amount of cardiac fibrosis was quantified by using the interactive imaging analysis system Quantuepatho as described previously.[11] This computer program allows the quantification of myocardial fibrosis on the basis of Masson's trichrome stained tissue. After the cardiopathologist (J.M. and K.K.) has defined fibrosis on each tissue section the computer program automatically calculates the fibrous tissue on the basis of the specific blue stained areas (defined directly by the pathologist) and converts it in green areas as described in **Figure 1A and B**. The results are given as the collagen volume fraction (CVF) in area percentage (%) of fibrosis in relation to the total area of the biopsy containing tissue.

Table S1. Impact of a 'restrictive' LV response to inotropic stimulation.

	Improved diastolic function with dobutamine		Impaired diastolic function with dobutamine		*p* Value Baseline	*p* Value Dobutamine
Patients, n	15		10			
Sex, male/female	12/3		8/2			
BSA, m^2	1.66 ± 0.29		1.71 ± 0.38		0.69	
Age at study, years	18.8 ± 4.7		21.6 ± 8.5		0.29	
Age at HT, years	6.3 ± 6.3		12.0 ± 12.6		0.22	
Follow-up since HT, years	12.5 ± 6.6		9.6 ± 6.4		0.29	
Acute rejection (≥2R), patients, n (%)	9 (60)		4 (40)			
Acute rejection (≥2R), episodes, n	13		5			
Donor ischemic time, min	227 ± 63		201 ± 77		0.36	
NYHA functional class, I/II/III	12/3/0		4/4/2		**0.04**	
BNP, pg/ml	34 (10-416)		48 (16-261)		0.61	
CMR						
LV mass, g/m2	54 ± 12		63 ± 14		0.10	
LV mass-to-volume ratio	1.26 ± 0.35		1.14 ± 0.30		0.39	
LV free wall T1 time native, ms	1239 ± 35		1324 ± 82		**0.05**	
Septal T1 time native, ms	1220 ± 42		1288 ± 88		**0.02**	
Catheterisation						
CAV class, 0/-1/-2/-3, n	6/7/1/0		3/6/1/0		0.84	
Conductance study						
Heart rate, /min	82 ± 10	107 ± 17	81 ± 11	104 ± 12	n.s.	n.s.
CI, l/min/m^2	3.69 ± 0.63	4.62 ± 1.08	4.30 ± 0.73	4.81 ± 0.91	**0.04**	n.s.
Pes, mmHg	91 ± 12	132 ± 23	90 ± 13	140 ± 26	n.s.	n.s.
Ped, mmHg	7.4 ± 4.1	6.7 ± 5.9	7.2 ± 4.7	6.5 ± 7.1	n.s.	n.s.
LVEDVi, ml/m^2	68 ± 13	67 ± 15	80 ± 17	69 ± 15	**0.04**	n.s.
LVESVi, ml/m^2	23 ± 6	23 ± 7	26 ± 11	22 ± 6	n.s.	n.s.
LVSVi, ml/m^2	45 ± 8	44 ± 11	54 ± 10	47 ± 10	**0.02**	ns
LVEF, %	66 ± 3	65 ± 6	68 ± 8	68 ± 6	n.s.	n.s.
dp/dt max, mmHg/s	1397 ± 575	2377 ± 297	1158 ± 156	2456 ± 428	n.s.	n.s.
dp/dt min, mmHg/s	-1301 ± 386	-1882 ± 422	-1111 ± 191	-2028 ± 435	n.s.	n.s.
PRSW, mmHg	88 ± 43	102 ± 41	62 ± 35	98 ± 27	n.s.	n.s.
Eed, mmHg/ml/m^2	0.19 ± 0.10	0.11 ± 0.05	0.10 ± 0.08	0.13 ± 0.10	**0.01**	n.s.
Tau, ms	27 ± 4	22 ± 3	27 ± 3	21 ± 3	n.s.	n.s.
Ea, mmHg/ml/m^2	0.79 (0.48-1.25)	1.02 (0.63-2.78)	0.53 (0.37-2.82)	1.11 (0.52-4.03)	**0.03**	0.92
Ees, mmHg/ml/m^2	0.50 (0.20-1.82)	0.94 (0.20-2.25)	0.29 (0.11-2.52)	1.13 (0.26-5.10)	0.16	0.48
Ees, %	182 ± 107		452 ± 399		**0.02**	
Ea/Ees	1.75 ± 0.92	1.81 ± 1.37	2.37 ± 2.06	1.23 ± 0.70	0.72	0.40

Subgroup analysis between a group of patients with improved diastolic function (fall in end-diastolic pressure volume relation Eed, n=15) under dobutamine stress and those patients with an impaired response (rise or no change in Eed, n=10).

BSA, body surface area; HT, heart transplantation; NYHA, New York Heart Association functional class; BNP, b-type natriuretic peptide; CMR, cardiac magnetic resonance; CAV, coronary allograft vasculopathy; CI, cardiac index; LV, left ventricle; EDV, enddiastolic volume; ESV, endsystolic volume; SV, stroke volume; EF, ejection fraction; Ped, end-diastolic pressure; Ped, end-systolic pressure; Tau, time constant of isovolumic relaxation; dP/dt max/min, maximum and minimum rate of pressure change in the ventricle; PRSW, preload recruitable stroke work; Eed, enddiastolic pressure volume relation; Ea, systemic arterial elastance; Ees, ventricular elastance; Ees %, percentage change in Ees with dobutamine; Ea/Ees, ventriculo-arterial coupling.